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Involvement of D1 and D2 dopamine receptors in the antidepressant-like effects of selegiline in maternal separation model of mouse

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HIGHLIGHTS

- Maternal separation (MS) provoked depressive-like behaviors
- Selegiline exerted antidepressant-like effects in MS mice
- Effect of selegiline on the passive behaviors is mediated via D1 receptor
- D2 receptor mediated effect of selegiline on the hedonic difficulties
- Effect of selegiline on the self-care problems is mediated via both D1 and D2 receptors

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ABSTRACT

Mother-infant interactions are known to be associated with the psychological well-being of an individual in adulthood. It is well accepted that emotional stress in early life, such as maternal separation (MS), leads to alterations in the neurotransmission systems of various brain regions, especially the mesolimbic dopaminergic system, and subsequently can increase the risk for development of psychiatric disorders including depression in adulthood. Selegiline is an irreversible monoamine oxidase (MAO) type B inhibitor which increases striatal dopamine levels and exerts an antidepressant effect. In this study, 180 min of MS stress was applied to mice at postnatal day (PND) 2–14 followed by behavioral tests for determining depressive-like behaviors, such as forced swimming test (FST), splash test and sucrose preference test (SPT) in adult mice (PND 50). The open field test (OFT) also was applied to validate FST results. We used SCH23390 (D1 antagonist) and sulpiride (D2 antagonist) in order to determine the role of D1 and D2 dopamine receptors in antidepressant-like effects of selegiline. Our results revealed that MS provoked depressive-like behaviors in adult male mice, and the administration of selegiline attenuated depressive-like behaviors in MS mice. Our findings showed that D1 dopamine receptors facilitate the positive effects of selegiline on the passive behavior in the FST. Furthermore, antidepressant effects of selegiline on hedonic difficulties are mediated via D2 receptor in the SPT. The results of the splash test revealed that both D1 and D2 receptors mediate the protective effect of selegiline against motivational and self-care problems. Based on our results, we conclude that both D1 and D2 dopamine receptors are involved in mediating the antidepressant-like effect of selegiline. We found that D1 receptors mediate an effect on despair behavior, D2 receptors mediate an effect on anhedonia, and both D1 and D2 receptors contribute to the protective effects of selegiline on motivational complications.

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1. Introduction

Social environments, especially mother-infant synchrony during childhood, have a critical role in shaping the brain and behavior [1]. Previous studies have demonstrated that the quality of early life is strongly associated with psychological well-being in adulthood [2,3]. Adversity in the neonatal stage of life dramatically disturbs neuronal and brain development and subsequently affects an infant's physical and/or psychological health [4,5]. Growing body of evidence indicates that exposure to stress, especially during the neonatal period, enhances the risk of neuropsychiatric states, including mood and depressive disorders in later life [6,7]. In this regard, it has been shown that experiencing early psychological stress such as maternal separation (MS) through alterations in neurotransmission in various regions of the brain induces psychiatric disorders, including depression [8–10].

Aversive emotional experience during infancy negatively impacts the structural and functional development of limbic brain circuits [11]. Maternal care, such as licking and grooming, stimulates the developing mesolimbic dopaminergic system which leads to an increase in dopamine levels as well as dopaminergic receptors [12]. In this regard, it is well-known that MS leads to neurotransmitter changes in the brain especially in the mesocorticolimbic dopamine neurotransmission [13–15]. Maternal separation stress has been shown to induce potentially enduring changes in the density of dopamine receptors as well as lasting derangements in dopamine neurotransmission in the brain [3,14]. Previous studies have demonstrated that MS affects dopaminergic circuits through an increase in levels of glucocorticoids (GC) [16,17].

Dopamine is an important neurotransmitter involved in a number of brain functions such as emotion, reward and behavior, as well as the neuropsychiatric disorders such as depression [18] [19,20]. Depressive disorder is a debilitating mental disorder with high prevalence, morbidity, and costly socioeconomic burden [21]. Anhedonia and motivational impairments are major symptoms of depressive disorder which are associated with reward pathway deregulations. It has been shown that dopamine is associated with motivation, reward and hedonia states. Therefore, increasing dopamine levels may be considered as an avenue for the treatment of depression [19,22,23]. It has been well established that dopamine agonists improve depressive symptoms [24]. In this regard, ample evidence suggested that dopamine D1 receptor mediated the effects of antidepressant drugs in the forced swim test (FST), while other studies demonstrated that dopamine D2 receptor antagonists blocked the effect of antidepressant drugs in the FST [25,26].

Selegiline [(−)-deprenyl] is an irreversible monoamine oxidase (MAO) type B inhibitor, an enzyme involved in dopamine metabolism [27]. It has been shown that selegiline increased striatal dopamine levels, and significantly improved a whole variety of depressive symptomatologies [28]. Since selegiline is metabolized to (−)-methamphetamine and (−)-amphetamine, the effects of drug on dopamine levels in the brain may be due to its amphetamine-like action [29]. Considering that selegiline possesses antidepressant effects and also potentiates the effects of antidepressants, there could be possible uses for selegiline in the management of resistant depression [30–32].

Although selegiline has antidepressant effects by increasing the striatal dopamine levels, role of D1 and D2 dopaminergic receptors are ambiguous in these effects. Using MS paradigm, this study aimed to explore the role of D1 and D2 receptors in the behavioral tests relevant to depressive-like behaviors in male mice.

2. Materials and methods

2.1. Animals

Pregnant NMRI mice (Pasteur Institute, Tehran, Iran) were used in this study. Animals were maintained under standard laboratory conditions (12-h light/dark cycle, temperature (22 ± 1 °C) and free access to food and water). The day of birth was considered as postnatal day 0 (PND 0). The litters were subsequently assigned to the MS paradigm. In this regard, pups were briefly handled and separated from their mothers for 180 min daily during PND 2 to PND 14, beginning at 09:00 a.m. [33,34]. At the end of the separation period pups were returned to the nest cage. At PND 21, male offspring were housed in groups until experiment day PND 50.

All procedures in this study were carried out in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication # 80-23) and institutional guidelines for animal care and use (Department of Pharmacology, School of Medicine, TUMS). All experiments were conducted between 10:00 a.m. and 02:00 p.m. Each experimental group consisted of 6 to 8 animals.

2.2. Drugs

The drugs used in this study were as follows: 1) selegiline hydrochloride: MAO-B inhibitor, 2) sulpiride (SULP): dopamine D2 receptor antagonist, and 3) SCH 23390 (SCH): dopamine D1 receptor antagonist. All drugs were purchased from Sigma, St Louis, MO, USA. All drugs were dissolved in 0.9% saline in a volume of 10 ml/kg. SULP and SCH were administered intraperitoneally (i.p.), whereas selegiline hydrochloride was administered via subcutaneous route (s.c) injections. Doses of each drug were selected based on previous published studies by Binfaré et al. and Shimazu et al. [25,35] and our own pilot studies. We treated mice with selegiline hydrocloride (60 min), SULP and SCH (90 min) prior to behavioral experiments. Dosing calculations were determined using the weight of the compound (including its salt). The dose of each drug was adjusted according to animal body weight (mg of drug/kg of body weight of mice).

2.3. Study design

Behavioral experiments consisted of open field test (OFT), forced swimming test (FST), splash test and sucrose preference test (SPT) which were conducted using control or MS mice as follows:

Experiment 1 tested the effects of the early stress experience, MS, on the different behavioral tasks related to assessing depressive-like behaviors.

Experiment 2 examined the effect of selegiline hydrochloride (1, 3, and 5 mg/kg) on depressive-like behaviors using behavioral tasks. Animals in this experiment received selegiline hydrochloride 60 min before the behavioral tests.

In experiment 3, we examined the effect of selegiline hydrochloride (3 mg/kg) along with SCH (0.03 mg/kg) or SULP (50 mg/kg) on depressive-like behaviors by using behavioral tasks. Animals in this trial received SCH or SULP 30 min prior to selegiline injection and 90 min before the tests.

2.4. Forced swimming test (FST)

The FST was carried out according to the previously described method by Porsolt et al. and Cryan et al. [36,37]. In this behavioral test, extended immobility time represents despair behavior reflecting depressive-like symptoms. Mice were separately placed in an open cylinder-shaped flask (diameter 10 cm, height 25 cm), containing 19 cm water at 23 ± 1 °C. Mice were permitted to swim for 6 min and the period of immobility was recorded throughout the last 4 min of the test. Each mouse was judged to be immobile when it ceased struggling and stayed floating motionless in the water and making only those movements necessary to keep its head above water.

2.5. Open field test (OFT)

The OFT was used to illuminate the effects of MS and treatments on motor function, exploratory behavior, and to rule out possible alterations in locomotion that might affect FST [38]. The OFT apparatus

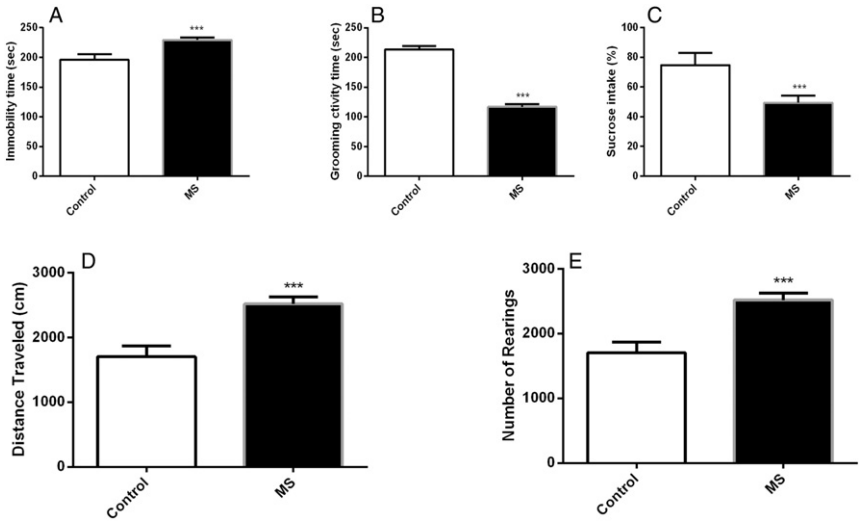

Fig 1. Effects of MS on the immobility time in the FST (A), grooming activity in the splash test (B), the percentage of sucrose consumption in the SPT (C), and the horizontal activity (D) and vertical activity (E) in the OFT. Values are expressed as the mean ± S.E.M from 6 to 8 animals and were analyzed using *t*-test. ***$P < 0.001$ compared with the control mice.

was made of white opaque Plexiglas (50 cm × 50 cm × 30 cm) which was dimly illuminated. Each mouse was placed gently on the central zone (30 cm × 30 cm) and its behaviors were recorded by a camera for 5 min and analyzed by Ethovision software version 8 (Noldus, Netherlands). The distance moved (horizontal activity) and the number of rearings (vertical activity) were evaluated. The apparatus was cleaned with 70% ethanol after testing each mouse.

2.6. Splash test

Splash test was used to assess motivational and self-care difficulties, which indicates depressive-like behaviors in animals [39]. Lack of motivation and self-care behaviors, as the symptoms of depression, is determined by a reduction in grooming activity time. In this behavioral test, the grooming activity of mice including nose/face grooming, head washing and body grooming, which considered as an indirect measure of pleasant solution intake, was recorded. A 10% sucrose solution was spurted on the dorsal coat of animals in their home cage and was animals were filmed for 5 min. The total grooming activity time was recorded during 5 min after the sucrose vaporization.

2.7. Sucrose preference test (SPT)

The SPT was applied to estimate the hedonic state in animals following a protocol previously described by Wallace et al. [40]. In this regard, two bottles of tap water were introduced to animals by placing one bottle in the home cage of each mouse for the first two days. Then, for the second two days one of the bottles was replaced by a bottle containing 1% sucrose solution. On the test day, animals were deprived of food and water for five hours and then sucrose preference was assessed during one hour of liquid consumption using two bottles in each cage, one of 1% sucrose solution and one of tap water. SPT was measured using the following equation which evaluates the ratio of 1% sucrose solution consumed to the total liquid consumed: sucrose preference = sucrose consumed / (sucrose consumed + tap water consumed).

2.8. Statistical analysis

Comparison between groups was analyzed using *t*-test and one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant. The sample size was calculated by power calculations using G power software (ver.3.1.7, Franz Faul, Universitat Kiel, Germany). We set α error at 0.05 and power (1-β) at 0.8 and the required total sample size per group was calculated as 6–8 in behavioral tests. We also calculated the power value in each experimental group and analyses have shown that the power values were larger than 0.8 in all ANOVA analyses. The effect size for the both ANOVA and *t*-test analyses were calculated by the Cohen's formula [41].

3. Results

3.1. Effects of MS on the depressive-like behaviors

Our *t*-test analysis revealed that the MS paradigm induced depressive-like behaviors in MS mice when compared to control animals. In the FST, MS mice showed an increase in the immobility time in comparison with normal animals ($P < 0.001$, Fig. 1A, effect size: 4.48). Results show that MS caused a significant reduction in grooming activity time in the splash test when compared to control animals ($P < 0.001$, Fig. 1B, effect size: 18.302). Furthermore, SPT findings show that, MS significantly reduced sucrose consumption in comparison with control group ($P < 0.001$, Fig. 1C, effect size: 3.821). In the OFT, MS significantly increased the total distance moved (horizontal activity) ($P < 0.001$, Fig.1D, effect size: 5.864) and number of rearings (vertical activity) ($P < 0.001$, Fig.1E, effect size: 5.864) in comparison to control mice.

3.2. Effects of selegiline on the depressive-like behaviors in animals

In order to determine the effective dose of selegiline, we assessed the effects of various doses of selegiline (1, 3 and 5 mg/kg, s.c.) on depressive-like behaviors in both MS and control mice using the above-mentioned tests. One-way ANOVA analysis demonstrated that treatment with selegiline (5 mg/kg) produced significant alterations in depressive-like behaviors in control mice when compared with saline-treated control mice in the FST ($P < 0.001$, Fig. 2A, effect size: 0.902), splash test ($P < 0.001$, Fig. 2B, effect size: 0.982), and in the SPT ($P < 0.05$, Fig. 2C, effect size: 0.870). However, doses of 1 and 3 mg/kg of selegiline had no effect in the aforementioned behavioral tests in control mice. In addition, selegiline (1, 3 and 5 mg/kg) produced no significant changes in horizontal and vertical activities in the OFT in control mice (Fig. 2D, effect size: 0.893 and Fig. E, effect size: 0.798). One-way

ANOVA analysis revealed that unlike dose 1 mg/kg, selegiline in doses 3 and 5 mg/kg significantly changed the depressive-like behaviors in MS mice in the FST($P < 0.001$ and $P < 0.001$, Fig. 2A), splash test ($P < 0.001$ and $P < 0.001$, Fig. 2B), and SPT ($P < 0.05$ and $P < 0.01$, Fig. 2C). In the OFT, Tukey's post-hoc analysis showed that administration of selegiline (3 and 5 mg/kg, but not 1 mg/kg) to MS mice significantly decreased horizontal activity in comparison with saline-treated MS mice ($P < 0.01$ and $P < 0.01$, Fig. 2D). However, MS mice did not significantly respond to selegiline (1, 3 and 5 mg/kg) in vertical activity in OFT.

3.3. Anti-depressant effects of selegiline mediated via D1 and D2 dopamine receptors

One-way ANOVA analysis revealed that treatment of MS mice with selegiline (3 mg/kg) significantly reduced the immobility time in FST in comparison with saline-treated MS mice ($P < 0.001$, Fig. 3A, effect size: 0.892). Co-administration of SCH (0.03 mg/kg) with selegiline (3 mg/kg) in MS mice significantly increased the immobility time in comparison with saline-treated counterpart ($P < 0.05$). The administration of SULP (50 mg/kg) plus selegiline significantly decreased the immobility time when compared to saline + MS counterpart ($P < 0.001$). The results of the FST revealed that unlike SULP + selegiline, pretreatment with SCH significantly reversed the effect of selegiline on the immobility time in comparison with selegiline- treated MS group ($P < 0.001$). Splash test results show that selegiline (3 mg/kg) significantly increased the grooming activity time in MS mice in comparison with saline-treated mice ($P < 0.001$, Fig. 3B, effect size: 0.985). Co-administration of SULP with selegiline significantly decreased the grooming activity time in comparison with saline-treated MS counterpart ($P < 0.01$). The findings of the splash test showed that pretreatment with SCH or SULP significantly reversed the effect of selegiline on the grooming activity time in comparison to selegiline- treated MS mice ($P < 0.001$ for both). Also, when compared with the saline-treated MS mice, we found that selegiline (3 mg/kg) significantly increased sucrose consumption in the MS group ($P < 0.05$, Fig. 3C, effect size: 0.900). Co-administration of SULP with selegiline significantly decreased sucrose intake in MS mice when compared to saline-treated MS mice ($P < 0.05$). The results of SPT showed that co-administration of SULP with selegiline significantly decreased sucrose consumption when compared to selegiline-treated MS mice ($P < 0.001$). One-way ANOVA analysis demonstrated that treatment with selegiline (3 mg/kg) alone or in combination with SCH and/or SULP produced no significant alterations in depressive-like behaviors in control mice in comparison to saline-treated or selegiline-treated control mice in the FST, splash test and SPT.

Furthermore, Fig. 3D (effect size: 0.824) and Fig. 3E (effect size: 0.804) show that treatment with selegiline (3 mg/kg) alone or plus SCH and/or SULP produce no significant changes either in horizontal or vertical activities in the OFT when compared to their saline/selegiline-treated counterparts in both MS and control mice.

4. Discussion

The results of the present study showed that MS, as an accepted paradigm of early life stress, provoked behaviors relevant to depression in

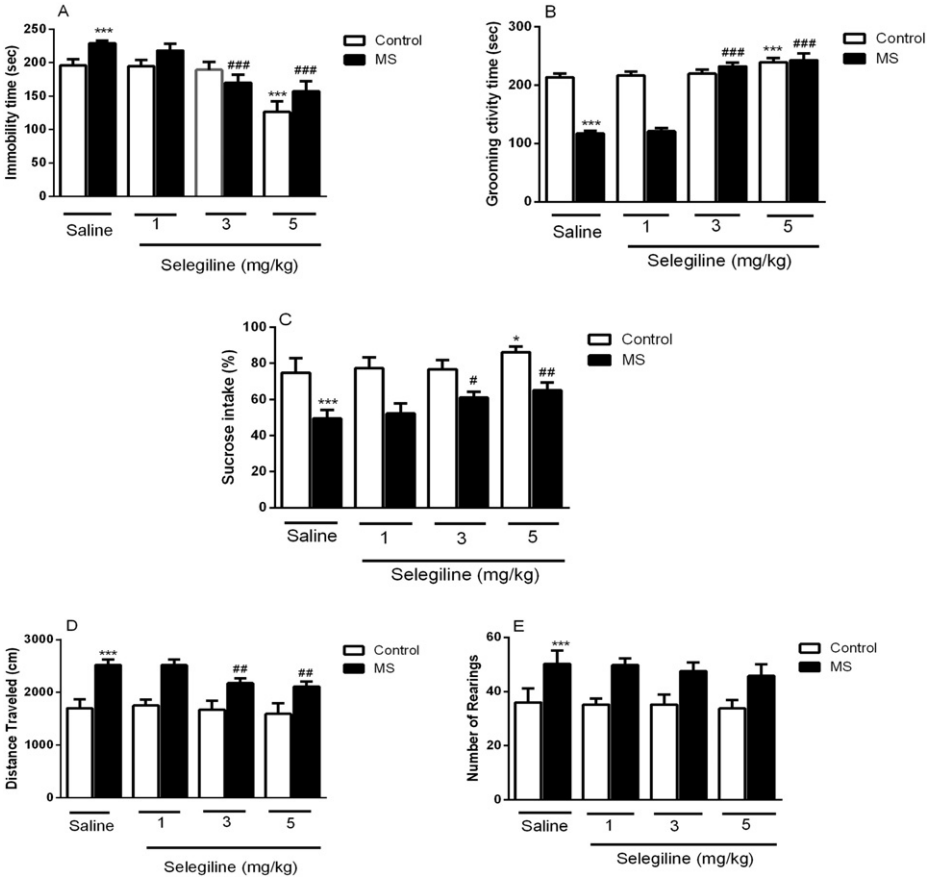

Fig. 2. Effect of selegiline (1, 3, and 5 mg/kg) treatment on the immobility time in the FST (A), grooming activity in the splash test (B), the percentage of sucrose consumption in the SPT (C), and the horizontal activity (D) and vertical activity (E) in the OFT in MS and control animals. Values are expressed as the mean ± S.E.M from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$ and $^{***}P < 0.001$ compared with the saline-treated control mice, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with the saline-treated MS mice.

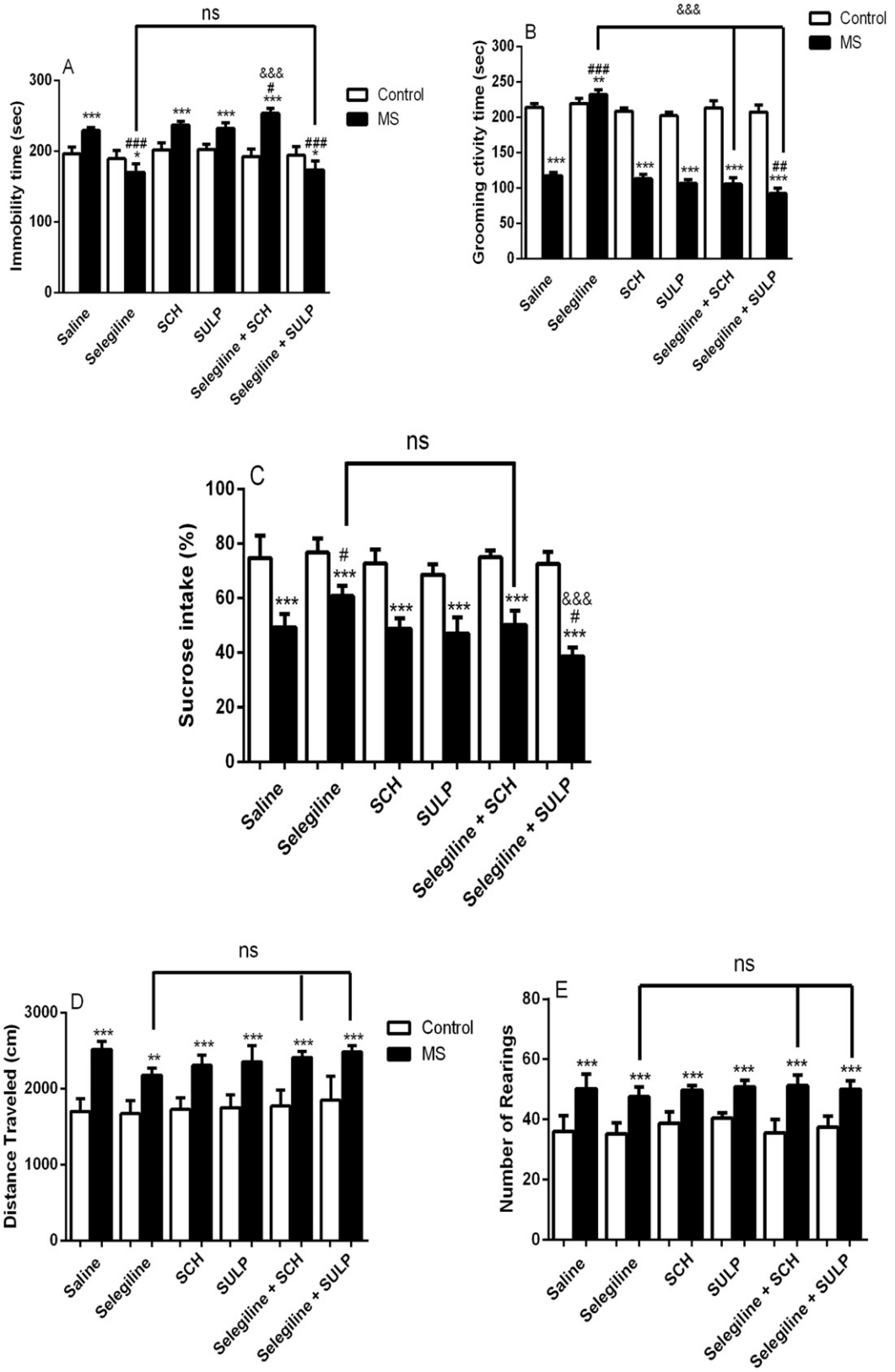

Fig. 3. Involvement of D1 and D2 dopamine receptors in antidepressant effects of selegiline on the immobility time in the FST (A), grooming activity in the splash test (B), the percentage of sucrose consumption in the SPT (C), and the horizontal activity (D) and vertical activity (E) in the OFT in MS and control animals. Values are expressed as the mean ± S.E.M from 6 to 8 animals and were analyzed using one-way ANOVA followed by Tukey's post hoc test. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the saline-treated control mice, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with the saline-treated MS mice, $^{\&\&\&}P < 0.001$ compared with the selegiline (3 mg/kg)-treated MS mice.

later life. These depressive-like behaviors were attenuated following selegiline treatment. Furthermore, our results showed that the antidepressant effects of selegiline on passive behaviors in the FST may be mediated via D1 receptors. Further, we found that D2 receptors mediate the effects of selegiline on motivation, self-care behaviors and hedonic state.

Maternal–newborn contact has a key role in the organization of an infant's physiological systems as well as development of the brain. Ample evidence indicates that early maternal contact is associated with bio-behavioral development which stimulates shaping of physiological and behavioral processes, and enhances social adaptation [42–44]. In contrast, previous investigations have demonstrated that early maternal separation causes enduring negative effects on brain and behavior [45]. Clinical studies have reported that maternal care may be considered as a parameter for prediction of social maturity in adolescence among children at risk of depression [46]. In this study, our results demonstrated that experiencing MS stress during P2-P14 is associated with the development of depressive-like behaviors in adult male NMRI mice. It is important to note that there are several factors that can affect the results following an MS paradigm. In this regard, there is evidence indicating that strain differences and different MS paradigms are able to change experiment outcomes [47,48]. As stated above, we applied a MS paradigm that has been shown to induce behavioral abnormalities in animals in previous studies [33,34]. Further, we used NMRI mice in this study primarily because results obtained in our laboratory and others have shown that this strain is sensitive to effects of social stressors such as adolescent social isolation, social defeat, maternal separation, and chronic mild stresses [49–54].

A large body of evidence has demonstrated that MS stress is able to alter neurotransmission in the various regions of the brain, and these changes are associated with the development of mood and anxiety disorders [8–10]. The results of our study are in line with previous reports indicating that the MS paradigm negatively evokes behaviors relevant to depression [5,7]. In this context, our behavioral testing showed that MS mice exhibited an increase in immobility time, as a passive behavior in the FST, similar to behavioral despair as a core symptom observed in human depression [37]. The splash test is an accepted method evaluating the self-care difficulties and motivation in rodents [55]. MS mice showed a poor response to sucrose 10% (decrease in the grooming time) indicating a disruption in motivation and self-care behaviors. It is well established that SPT is a valid test for evaluating the anhedonia in animals [40]. In this regard, our findings were validated by development an anhedonic state (reduction in sucrose consumption) of MS mice in the SPT indicating that MS is able to induce anhedonia in animals [56]. OFT was applied immediately before the FST to evaluate ambulatory behavior of mice and also confirm that variations which occur in motor activity did not affect the duration of immobility [57]. In the OFT, MS mice showed an increase in locomotion in both vertical and horizontal activities. In the case of OFT, it should be noted that there is an inconsistency in the literature regarding OFT results following MS paradigm. In this regard, previous studies reported that MS decreases locomotion [48], increases locomotion [58] or do not change ambulation in the OFT [59].

While there are concerns about the cogency of FST, this test is a valid and rapid screening test for assessment of new antidepressant agents. It has been well established that depressed patients have difficulties dealing with stressful conditions and, similarly, depressed rodents behave the same in the stressful conditions (cylinder filled with water) by exhibiting passive behaviors [37,60]. In this study, we also applied the splash test and SPT as valid tools to assess motivation and self-care behavior as well as hedonic state in animals [55,61]. Moreover, an increase in the number of rearings (vertical activity) and distance traveled (horizontal activity) by MS mice in the OFT validated our FST results.

Experiencing psychological adversity in infancy has a negative influence on the development of mesolimbic dopaminergic system [16,52,62–64]. Evidence is accumulating that MS leads to neurotransmitter changes in the brain. In this regard, it has been shown that MS induces long-lasting alterations in the dopaminergic system of the central nervous system [65–67]. Dopamine is an important monoamine involved in emotion, reward and behavior as well as depression [19]. Hedonia and motivational impairment along with despair behavior are major symptoms of depressive disorder which are associated with dysfunction of the reward pathway [68,69]. Regarding the involvement of dopamine in motivational and hedonic behaviors, those agents which are capable of enhancing dopaminergic activity may potentially have antidepressant properties [70].

On the other hand, it is well determined that selegiline [(−)-deprenyl], an irreversible monoamine oxidase (MAO) type B inhibitor, increased striatal dopamine levels and possess antidepressant activity [28,31]. In this context, our results showed that administration of selegiline improved depressive-like behaviors. Following treatment with selegiline in MS mice, the immobility time decreased in the FST, grooming time increased in the splash test as amount of sucrose intake enhanced in the SPT. OFT revealed that selegiline decreased the horizontal activity of MS mice.

In order to determine the role of D1 and D2 dopaminergic receptors in antidepressant effects of selegiline, using SCH (D1 antagonist) and SULP (D2 antagonist) we showed that co-administration of SCH with selegiline increased immobility time in MS mice, while no change was observed in the splash test and SPT. This indicates that the antidepressant effects of selegiline on passive behavior in the FST are mediated via D1 receptor. Our findings show that blocking of D2 receptors by pretreatment with SULP reversed the effects of selegiline in the splash test and SPT in MS mice. This suggests that the effect of selegiline on the motivational and self-care behaviors as well as hedonic state is mediated through D2 receptors. Previous studies have demonstrated that D1 receptors mediate the antidepressant effects of drugs in the FST, whereas D2 receptor antagonists are reported to have an antagonistic effect on the antidepressant like effect of drugs in the FST [25,26]. Our results are in line with studies that demonstrated that D2 receptor is not associated with behaviors in the FST, but are associated with hedonic and motivation behaviors [25,71]. Furthermore, both D1 and D2 receptor antagonists have been reported to antagonize the therapeutic effects of antidepressants [72–74]. In this regard, a recent study by Li et al. demonstrated that dopamine receptors contribute to the rapid antidepressant-like effects of ketamine in the FST [75]. In addition, the acute antidepressant-like effects of selegiline have been reported in the FST. In this context, Shimazu and colleagues reported that the acute antidepressant-like effects of selegiline in the FST is associated with activation of D1 receptors and not MAO-B inhibition. Also, they demonstrated that acute effects of selegiline may be related to its main metabolite (−)-methamphetamine. Our results are in agreement with this previous study and demonstrate that acute effects of selegiline are mediated by dopaminergic system [25].

The antidepressant effects of selegiline have been well documented in the literature, however there is no information on the contribution of D1 and D2 dopamine receptors on the antidepressant effect of selegiline [30,76]. In the current study, we have shown that D1 receptors mediate the antidepressant-like effect of selegiline in the FST (passive behavior). Moreover, effects of selegiline on self-care behavior and hedonic state in the splash test and SPT are mediated through D2 receptor. Indeed, MS stress is associated with poor development of reward pathways, specifically in male pups that makes them susceptible to many challenges in later life such as addictive behaviors as well as psychiatry disorders. On the other hand, selegiline is known to improve the performance of dopaminergic system in people with Parkinson's disease. Because this compound is actively converted to L-amphetamine in the body, we hypothesized that maybe the antidepressant effects of selegiline is associated with its ability to enhance the activity of dopaminergic system, which specifically are affected by MS stress. After results of this study, we also applied chronic selegiline to maternally separated rats during their adolescent period, and we observed interesting results and we

found advantageous effects of selegiline are associated with its effects on mitochondrial function and other parameters in the brain. However, in case of behavioral tests, we observed that effects of selegiline are mediated by dopaminergic system in a specific manner (unpublished data).

5. Conclusion

In conclusion, the results of our study show that: 1) experiencing MS in early life provoked depressive-like behaviors in adult male mice, 2) selegiline exerts antidepressant properties in MS mice, 3) D1 dopamine receptors facilitate the positive effect of selegiline on despair behavior in the FST, 4) the antidepressant effects of selegiline on motivational difficulties may be mediated via both D1 and D2 receptors in the splash test, and 5) D2 receptors may be involved in the protective effects of selegiline against anhedonia problems in the SPT.

Conflicts of interest

The authors have no conflicts of interest to declare regarding the study described in this article and preparation of the article.

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